An anonymous DNA probe E141 (D5S99) on chromosome 5 detects an EcoRI polymorphism

L.Warnich*, E.Dietzsch, J.S.Herbert, J.Brusnický, L.Laubscher and A.E.Retief
MRC Cytogenetics Research Unit, Faculty of Medicine, PO Box 63, Tygerberg 7505, South Africa

Source and Description: A 13.8 kb fragment cloned into the EcoRI site of phage lambda L47.1, isolated from a human genomic library. The insert has an internal EcoRI site which divides it into two fragments (8.4 and 5.4 kb).

Polymorphism: EcoRI identifies a two allele polymorphism (A1: 4.5 kb; A2: 5.4 kb) with constant fragments at 8.2 and 2.05 kb.

Frequency: Studied in 65 parents of CEPH families.
A1: 0.53
A2: 0.47

Not Polymorphic For: TaqI and RsaI with a panel of 5 unrelated individuals.

Chromosomal Localization: The probe was assigned to chromosome 5 using a panel of somatic cell hybrids and localized to 5q12 by means of in-situ hybridization.

Mendelian Inheritance: Co-dominant segregation has been observed in 28 informative CEPH families.

Probe Availability: Available for collaboration.

Other Comments: The probe was pre-reassociated with an excess of sonicated total human DNA prior to hybridization.

Acknowledgements: The work was supported by the Medical Research Council, Cape Provincial Administration and the University of Stellenbosch.

* To whom correspondence should be addressed

Human ApoCI HpaI restriction site polymorphism revealed by the polymerase chain reaction

W.M.Nillesen, H.J.M.Smeets and B.A.van Oost*
DNA-Diagnosis Laboratory, Institute of Human Genetics, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands

Source/Description: Klasen *et al.* (1) described a HpaI site polymorphism that is located at the 5′ end of the ApoCI gene. Using the published sequence data (2) we have selected primers to amplify a 222 bp fragment encompassing the polymorphic HpaI restriction site.
5′-primer upstream: 5′-TTT GAG CTC GGC TCT TGA GAC AGG AA-3′. *3′-primer downstream*: 5′-GGT CCC GGG CAC TTC CCT TAG CCC CA-3′.
Protocol: The PCR was performed using 1 μg of DNA and 10 pMoles of each primer. DNA was amplified for 30 cycles using a New Brunswick thermal cycler: denaturation: 50 sec 95°C, annealing: 50 sec 58°C, elongation: 90 sec 72°C. Ten μl of the reaction mixture was incubated with 20 U HpaI (Beckman Instruments Nederland) in 15 μl 20 mM potassium chloride buffer (pH 7.4). The reaction mixture was analysed directly on a 3.75% agarose, 0.25% NuSieve agarose (FMC BioProducts, Rockland, ME) gel run in 40 mM Tris-Acetate buffer (pH 8.0).

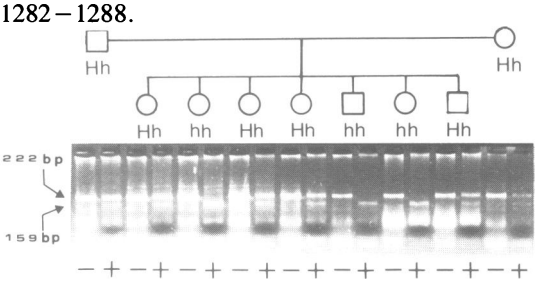
Polymorphism: Allele 1 (HpaI restriction site absent) is 222 bp and allele 2 (HpaI restriction site present) is 159 bp. The smaller fragment of 63 bp is usually not visible.

Frequency: Allele 1: 0.65, allele 2: 0.35.

Chromosomal Localisation: 19q13.2.

Mendelian Inheritance: Co-dominant segregation was observed in 12 myotonic dystropy families (see also figure). No recombinants between the ApoCI locus and the DM gene were observed in this set of families (Maximal LOD score of 6.6 at zero percent recombination).

References: 1) Klasen,E.C. *et al.* (1987) *Hum. Genet.* **75**, 244–247. 2) Smit,M. *et al.* (1988) *Biochem. Biophys. Res. Com.* **152**, 1282–1288.



Direct visualization of the amplification products before (−) and after (+) digestion with HpaI in an ethidium bromide-stained gel (see protocol). Both alleles are visible for the heterozygous parents (Hh), while their children are either heterozygous or homozygous for the smaller allele (hh).

* To whom correspondence should be addressed